Newsletter for November 2024

Integument update: “moar feather stuff”[1]

By Lathreas | November 30, 2024

We wrote some on feathers in our October update, and are going into additional details here as we continue working to sufficiently understand the systems.

Feathers are by far the most challenging integumentary structure to be trying to implement. For that reason, it requires extra deep review to understand first and foremost what they are shaped like, what variations exist in nature, and finally how they grow.

Crosstalk in the Wnt signaling pathway

There are a few highly conserved signaling pathways in animals that orchestrate the growth and patterning of structures. Several pathways keep popping up in our review: the Wnt pathway (often as an activator), the BMP pathway (often as an inhibitor), the ectodysplasin (EDA) pathway (as a wavefront guide), and the FGF pathway (often as an activator as well). Interestingly, the same pathways are re-used in different processes, hinting that differences in cell identity are also required to dictate how a cell responds to a signal, and that the signals themselves are just there to facilitate intercellular communication.

For this reason, it pays to take a close look at the various signaling pathways involved in the morphogenetic processes of fur, scale and feather development. Here we will focus on Wnt, due to its relevance in regulating size of hair follicles (Lei et al., 2014) but also due to their role in feathers (Li et al. from 2017) and scales (Tzika et al., 2023; Evanitski & Di Talia, 2023).

Beta-catenin is one of the main components of the canonical Wnt signaling pathway. However, its role is not limited to signaling in this pathway alone. Originally, it has been discovered as a component of adherens junctions by linking to cadherin (Ozawa et al., 1989), which is also present in skin. Somehow, the two functions of beta-catenin play out simultaneously (Valenta et al., 2012). Many researchers are focusing on this intriguing example of multifunctionality of proteins, and for us it’s of special importance: if there is

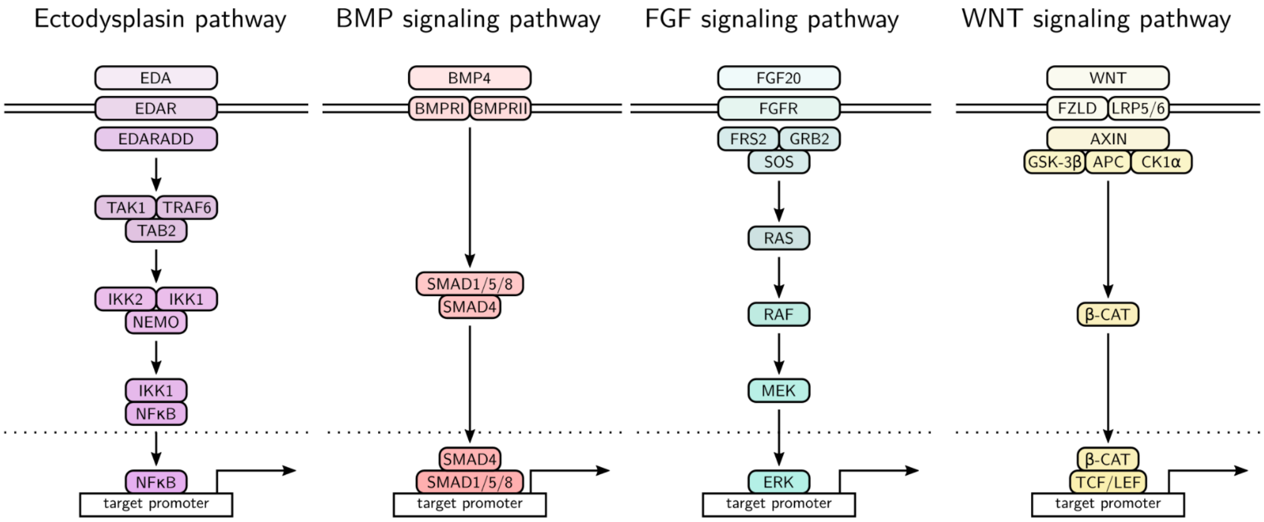

Figure 1: *simplified overview of various signaling pathways. Picture by Lathreas, shared under CC-BY-SA 4.0*

crosstalk between these two processes, we must understand how this impacts the growth of fur, scales, or feathers. There are many possible ways in which the two functions of beta-catenin may have an impact on one another, but there may even be processes to keep the two functions neatly separated. A little intuition for biological processes suggests that there may be a reason for any crosstalk to exist, for example by modulating the adherens junctions present in a cell in response to a signal. This latter effect has indeed been observed in the imaginal disks of *Drosophila melanogaster*, a.k.a. fruit flies, where the application of a Wnt11 signal has been shown to reduce the amount of beta-catenin bound to the membrane (or rather Armadillo, the beta-catenin homolog), and as such reduced cadherin-mediated cell adhesion (Wodarz et al., 2006). There are similarly some interesting observations in feathers that beta-catenin changes its structural organization in response to Wnt signaling, hinting that it may play a role more profound than just as a signal mediator (Shie et al., 2019). We'll come back to that at the end of this article!

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[1] Editor’s note: This title was in the draft presented to the editor. Editorial standards prevent changing it. :3

There is increasing evidence that beta-catenin-mediated cell adhesion and Wnt-signaling experience crosstalk (Bienz, 2005). This leads to the question of whether and how cells regulate these different functions of beta-catenin. There exist several important ways: one potential source of separation is the physical separation between freely floating beta-catenin in the cytoplasm and beta-catenin captured at the cell membrane. But even so, the two pools are intrinsically linked: if beta-catenin is sequestered to the membrane, for example when constructing new adherens junctions during the formation of structured tissue, beta-catenin necessarily must be withdrawn from the cytoplasmic pool. If indeed all beta-catenins are created equally, this is a potential source of crosstalk, since the same beta-catenin is also required for orchestrating gene regulation. Similarly, if cell junctions are destroyed, this may lead to a release of beta-catenin from the membrane and subsequent activation of Wnt-signaling target genes (Maretzky et al., 2005; Reiss et al, 2005). Interestingly, such an event can lead to a positive feedback loop, since in some scenarios, the freed beta-catenin downregulates genes related to cell junctions (Kim et al., 2019). Even so, there are several ways in which cells regulate the function of beta-catenins, such as through its various contextual post-translational modifications (Hoffmeyer, 2017; Valenta et al., 2012), as well as by modulating its available binding partners (Valenta et al., 2012), which all have the potential to alter the function of individual beta-catenins. Free-floating beta-catenin is targeted and destroyed by a destruction complex (Stamos & Weis, 2013); the canonical Wnt signaling pathway acts by inhibiting this destruction of beta-catenin and in this way changing the balance of cytoplasmic beta-catenin concentration. Overall, it is a highly intertwined and complex process, which must not be underestimated. A good review of the interplay between beta-catenin's two functions can be found in Valenta et al., 2012.

Many of these processes still have open questions, and each can have very context-dependent effects that may be unique to individual tissues and developmental stages. For fur, scale, and feather development specifically, this means that a Wnt signal given to one type of cell will not lead to the same response as a Wnt signal given to a different cell unless the inter- and intracellular contexts are sufficiently alike, and that is important to keep in mind.

Three different hypotheses for the growth direction of feather follicle barbs

With some nuances out of the way, we can take a closer look at how different signaling pathways work together. In order to understand how to reconstruct feathers *in vitro* for later transplantation, or whatever other method is being used to grow feathers, it helps to understand how all of the structures form. While working on the project, we discussed among ourselves and did deep-dive searches to verify the model we had covered back in 2021 by [Li et al. from 2017](), which proposed a specific mathematical model to explain feather branch formation. Although it is a compelling model, *it is easy to create mathematical models that behave similarly to real phenomena even when the real underlying mechanisms are totally different*. And here, too, there are still many open questions that weren't explained by the model, and alternative hypotheses that we ought to examine.

First and foremost, some basics: the feather follicle contains a base, which is proposed to 'print' cells in the direction facing out of the skin to produce the feather, quite akin to how hair grows. On this base plate, it is proposed that a wave-like pattern is formed through the interaction between Shh and BMP2 (Harris et al., 2005), in a process called Turing patterning. Naturally, when this pattern is subsequently extruded in the direction of feather growth, you will get nice stripes along the feather axis, which will become the barbs of a feather. However, when extruded like this, the stripes are all parallel to the feather stem, and don't connect anywhere! That means that you won't have a feather, but something more akin to a paintbrush. In order to connect the barbs to the stem of the feathers, the barb stripes grow at an angle in real flight feathers; which means there is some growth program orchestrating the stripes to grow at an angle.

The debate on how feather barbs grow at an angle is not yet fully settled, but is starting to show compelling evidence in one direction. There are three alternative hypotheses in the literature that give explanations for how feather barbs can grow at an angle: one by Harris et al., 2005, one by Yue, Jiang, & Widelitz, 2006, and one by Alibardi, 2009.

The first hypothesis, by Harris et al., proposes that cells at the base of the feather follicle stay stationary, and that due to the dynamics of the reaction-diffusion system of the BMP and Shh wave pattern, the waves move over time, along a GDF10 concentration gradient (Harris et al., 2005). Because the waves travel, each extruded layer will have a slightly shifted pattern imprinted on it, leading to an overall angled stripe pattern depending on the speed of extrusion and the speed of the traveling wave. This is a plausible explanation, because reaction-diffusion systems are known to be able to give rise to traveling waves. Yue, Jiang & Widelitz show that instead of GDF10, a WNT3a gradient may also be a candidate for orchestrating this process of wave movement (Yue, Jiang, & Widelitz, 2006). There is some evidence that the Wnt signaling here does not utilize beta-catenin and as such may act non-canonically, though more research is needed (Yue, Jiang, & Widelitz, 2006), though other work contradicts this claim (Li et al. from 2017). It is furthermore still unclear whether the pattern is formed purely on the base layer of cells, or whether pattern formation still proceeds further into the barb field. Finally, there is also the hypothesis that cells at the base physically translocate over the base plate towards the rachis, and in that way orchestrate the movement of the pattern. However, experiments done by Yue et al (2006) do not

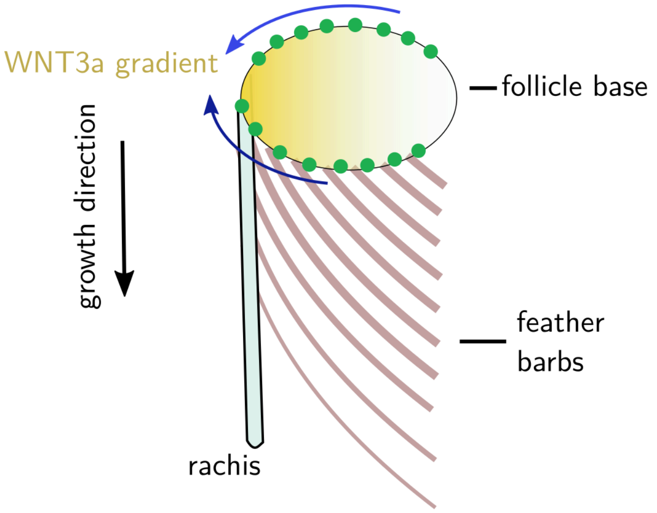


Figure 2: *A model of feather follicle growth, shown upside down to better highlight the base. In green: Shh expression pattern. In yellow: WNT3a expression pattern. In brown: barb patterns. Picture by Lathreas, shared under CC-BY-SA 4.0.*

support this hypothesis, and make it an unlikely candidate even if it is not yet fully ruled out.

A third hypothesis exists as well, by Alibardi (2009), where it is proposed that the barbs grow at an angle due to a physically angled growth plate and extrusion angle. Indeed, histological imaging has shown that the growth plate is indeed angled in real feather follicles (Alibardi, 2009). However, the hypothesis does not explain how the barbs subsequently get linked up to the feather stem, or what happens to barb stripes that loop around on themselves. Nevertheless, it will be important to take the angle of the follicle base into account, as this could drastically alter the dynamics of the system.

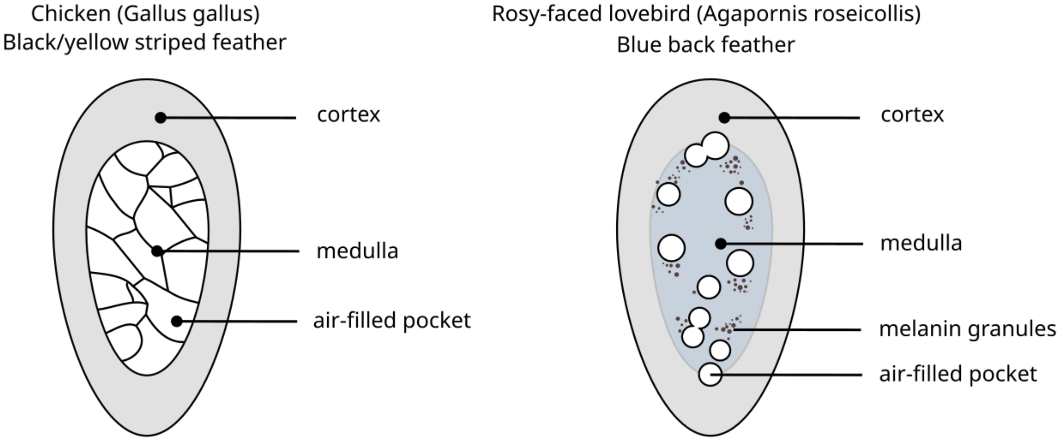
Overall, so far the evidence does seem to be in favor of the comprehensive model put forth by Li et al. in 2017, which is a good sign! Still, some questions remain before it's fully verified and usable for engineering purposes, and indeed the tilted base plate also needs to be taken into account. Although the model is based on experimentally found causations between different signal inputs and outputs, it has not been verified whether these occur in the exact proposed way at a molecular level, making it unclear if perturbations that we design based on the model will indeed have the desired effect. As such, what remains now is to find physical parameters that have not been hand-picked to give qualitatively correct results, but rather which have been experimentally measured. After all, anyone can hand-pick a parameter to get a model to work, but this is no guarantee that reality works in the proposed way. That said, finding said parameters is a very laborious task, and we will be optimizing for efficiently allocating our time so that we can engineer things, and as such, for now, this is where we will leave it for the sake of the review process.

There are several more things that need to be understood, such as how barbules form, and barbicels. These are the smaller sub-branches that together complete the velcro-like behavior of the vane of a feather, and these have seen less modeling efforts. Even so, some information does exist of their formation.

Some more feather structure stuff

For example, the cross-sectional morphology of ramus (the stem of a barb) shows how microstructures inside a barb's shaft are formed, which gives a hint of how morphogenesis occurs after the initial patterning. Pictured below is the cross-section of the ramus, showing how the cells collapse into hollow gaps in the case of chickens. In the case of rosy-faced lovebirds, however, the cells do not collapse fully, but instead the cell nuclei collapse to form spherical air-filled pockets. The remaining material is replaced by a fine tube-like network that gives rise to structural coloration, too small to be depicted here.

Figure 3: Ramus cross-sections for chicken (Gallus gallus) feathers (abstracted from Reddy & Yang, 2007) and rosy-faced lovebird (Agapornis roseicollis) (abstracted from Dyck, 1971).



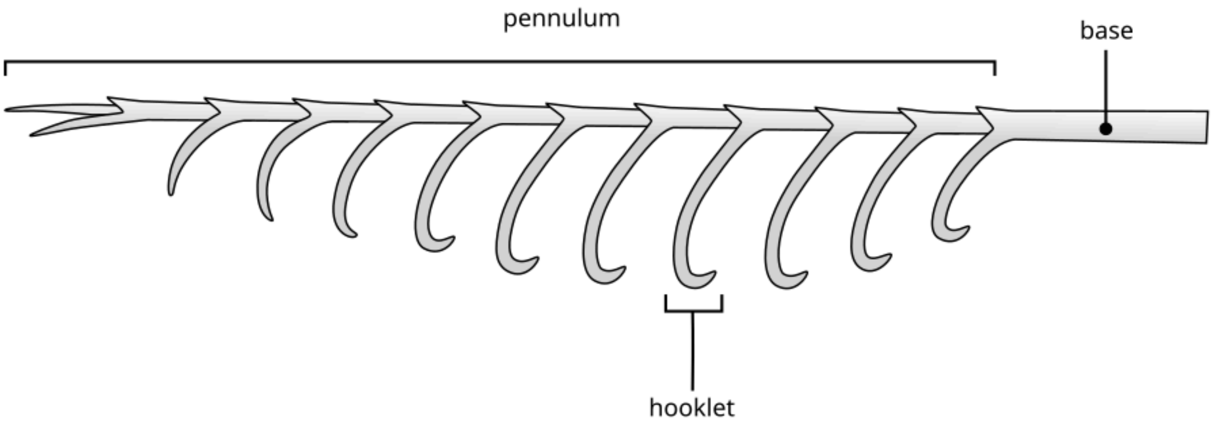


Figure 4: *Enlarged view of microscopic features of barbules. By Lathreas.*

Then finally, we can take a look at individual barbules — the branches that emanate from barbs — and find that each hooklet is just a single cell. We are currently diving into the molecular organization of such barbules, as well as how they develop.

Recent work by Shie et al. (2019) shows that Wnt2b, another Wnt protein, influences the barbule organization of a feather. They added Wnt2b beads to a developing feather, which did not significantly alter the barb organization, but did increase the amount of hooklet-like structures that formed (Shie et al., 2019). Furthermore, it increased expression of beta-catenin on the membranes of these structures, and changed the overall arrangement of beta-catenin to occur at more sides of each rectangular cell (Shie et al., 2019). Given what we know of the potential crosstalk between canonical Wnt signaling pathways and the structural role of beta-catenin, this leads to some very interesting questions and hypotheses that we will soon try to explore further.

So, all in all, feathers are *challenging* but also incredibly fascinating.

Thank you as always to everyone supporting us in the community, and thank you for reading!

For more detail on the Integument Review Project, check out our project page.

2nd head anatomy video is still cooking!

By Zennith | November 30, 2024

It's been a minute since we've checked in on the second head anatomy video! Honestly, for the past few months, I have been prioritizing helping the integument review project team wrap up their tissue-level descriptions of scales and then feathers. It's tricky, and based on everything we've been learning, engineering the biomolecular mechanisms in the integument might even be the key rate-limiter for us (so, we need to go go go on that project!)

Nevertheless, we are aware that anthros need anthro-shaped heads, so as to not just have human-shaped fuzzballs. :3

I took advantage of the holiday weekend in the US (Thanksgiving for our international folks) to get some focus work done. I cut several tangents out of the ongoing draft script, and reorganized and rewrote several sections.

The plan is largely the same as that I mentioned in our May 2024 newsletter, *but*, I figured out a much better way to present the concepts.

Previously, I was thinking of just having a section of the video dedicated to listing (effectively) each individual change, followed by a per-tissue-system crash-course in physiology and cell biology, followed by brief notes about what tissues might be possible to modify with various techniques.

That would have been…perhaps reasonable, but was on track to become, like, a 2-hour video, and be very dull to listen to.

I had the realization that I should just organize the entire video by tissue systems. For bones, for example, here's the flow that I decided on:

- Say what bones are, functionally, and tissues they interact or integrate with
- List only the parts of craniofacial bones that will be impacted by transformations
- Sneakily introduce tissue-level concepts alongside our commentary:
    - With the parietal and frontal bones, some of the simplest bones of the head, introduce osteons, lamellae, sutures, and the periosteum - describing why bone tends to be amenable to manipulation, but why we want to avoid disrupting sutures.
    - With the outer surfaces of the sphenoid, facing the temporalis muscle, introduce entheses (muscle/tendon attachment points) - and describe why we want to avoid disrupting entheses.
    - Around the nasopharynx and orbits, introduce what sinuses are, and describe bone manipulations that will be no big deal (including the vomer and perpendicular plate of ethmoid).
    - With the maxilla and mandible, go into more detail about entheses, and how we can extend relevant portions of those bones while avoiding unnecessary complexity.

A similar flow will be used for cartilage, muscles, a limited selection of internal or specialized tissues, and nerves. And of course, many anthropomorphic wolf 3D renders and vector illustrations will be used throughout!

In organizing the content like that, we can keep the entire discussion to only concepts that are actually relevant to modifying craniofacial anatomy. We're not writing an anatomy textbook, after all!

The point is to give an intuition of what needs to happen with each impacted tissue, and moreover, an understanding of which portions of the anatomy will be more, or less, amenable to modifications.